

Supplemental Text 1. CDC investigation into the folic acid calibration bias

CDC conducted a series of experiments that compared folic acid primary stock solutions of variable concentrations and age, folic acid intermediate stock solutions prepared in water vs. 0.1% ascorbic acid, and buffering of the daily calibrator mixture vs. using 0.1% ascorbic acid as the diluent. Our findings indicated problems with folic acid solubility at certain pH and concentration conditions.

According to Biamonte and Schneller, the folic acid solubility in an aqueous phosphate-citrate buffer at 30°C was highly pH dependent (pH 3, 4, 5, 6, and 7: 1–2 µg/mL, 10 µg/mL, 80 µg/mL, 2 mg/mL, 14 mg/mL, respectively) [1]. The folic acid solubility in water was also temperature dependent, but it was fairly constant at temperatures <50°C (~30–40 µg/mL) and increased to ~200 µg/mL at 95°C [1]. In the originally developed CDC method that measured 5-methylTHF and folic acid [2], the folic acid concentration of the primary stock solution was ~200 µg/mL phosphate buffer, pH 7.2 and the folic acid intermediate stock solutions were prepared in water. Later, when we incorporated additional folate forms into the method, we changed the diluent for the 10 µg/mL intermediate stock solution to 0.1% ascorbic acid (pH 3.6) to be consistent with what we used for the other reduced folate forms and we prepared the primary stock solutions at higher concentrations (~350–750 µg/mL). Furthermore, we prepared the daily calibrator mixture in 0.1% ascorbic acid, which did not provide appropriate buffering for the 0.5 M HCl present in the 5,10-methenylTHF calibrator. As a result of folic acid insolubility, the CDC folic acid calibrator was incorrectly value assigned (~30% too high), leading to an overestimation of serum concentrations by the CDC laboratory.

To avoid solubility problems, the primary folic acid stock solution should not exceed 200 µg/mL, the solvent pH should be neutral or alkaline, and the solution should be kept in a 28°C water bath for approximately 30 min to ensure dissolution. Intermediate folic acid stock solutions should be prepared in water and stored at -70°C. We found that intermediate folic acid stock solutions at 100 µg/mL prepared at neutral pH and stored at -70°C were stable for up to 10 y. The daily mixed calibrator should be prepared in a buffered solution.

To correct the folic acid calibration bias, CDC prepared a new calibrator and re-characterized a set of 23 in-house QC materials over a period of 11 days (folic acid concentration range: ~0.6–12 nmol/L). The new folic acid results were on average (SD) 25% (2%) lower. The weighted Deming regression equation was: Folic acid_{New} = 0.7586 * folic acid_{Original} – 0.016 nmol/L; 95% CI for slope: 0.7448 to 0.7724; 95% CI for intercept: -0.0337 to 0.0017; $R^2 = 1.000$.

References

1. Biamonte, AR, Schneller GH. Study of folic acid stability in solutions of the B complex vitamins. *J Am Pharmaceut Assoc* 1951;XL(7):313–20.
2. Pfeiffer CM, Fazili Z, McCoy L, Zhang M, Gunter EW. Determination of folate vitamers in human serum by stable-isotope-dilution tandem mass spectrometry and comparison with radioassay and microbiologic assay. *Clin Chem* 2004;50:423–32.

Supplemental Table 1. Folate HPLC-MS/MS method information¹

Laboratory #	Calibration range / LOD; type of internal standard used					
	5-MethylTHF	Folic acid	5-FormylTHF	THF	5,10-MethenylTHF	MeFox
10	1–100 / 0.13; ¹³ C ₅ -IS	0.5–50 / 0.20; ¹³ C ₅ -IS	0.5–50 / 0.20; ¹³ C ₅ -IS	0.5–50 / 0.25; ¹³ C ₅ -IS	0.5–50 / 0.20; ¹³ C ₅ -IS	0.5–50 / 0.10; ¹³ C ₅ -IS
<i>Group 1</i>						
1	0.02–100 / 0.06; ¹³ C ₅ -IS	0.02–100 / 0.08; ¹³ C ₅ -IS	Not measured	Not measured	Not measured	Not measured
2	8–70 / 0.07; ¹³ C ₅ -IS	1–50 / 0.27; ¹³ C ₅ -IS	1–50 / 0.52; ¹³ C ₅ -IS	Not measured	Not measured	3–54 / 0.20; ¹³ C ₅ -IS ²
3	5–165 / 0.8; D ₄ -IS ³	4.49–44.9 / 0.6; D ₄ -IS ³	3.52–87.9 / 1; D ₄ -IS ³	2.72–54.4 / 1; D ₄ -IS ³	Not measured	Not measured
4	0–32 / 0.5; ¹³ C ₅ -IS	0–32 / 0.5; D ₄ -IS ⁴	0–32 / 0.5; ¹³ C ₅ -IS	0–32 / 0.5; ¹³ C ₅ -IS	0–32 / 0.5; ¹³ C ₅ -IS ⁵	Not measured
5	0–100 / 0.10; ¹³ C ₅ -IS	0–100 / 0.20; ¹³ C ₅ -IS	0–100 / 0.09; ¹³ C ₅ -IS	0–100 / 0.90; ¹³ C ₅ -IS	0–100 / 0.15; ¹³ C ₅ -IS ⁵	Not measured
6	1.09–109 / 0.22; ¹³ C ₅ -IS	0.57–56.7 / 0.23; ¹³ C ₅ -IS	0.53–52.8 / 0.21; ¹³ C ₅ -IS	0.56–56.1 / 0.56; ¹³ C ₅ -IS	0.55–54.9 / 0.22; ¹³ C ₅ -IS	0.53–52.8 / 0.21; ¹³ C ₅ -IS
7	0–100 / 0.33; ¹³ C ₅ -IS	0–100 / 0.33; ¹³ C ₅ -IS	0–150 / 0.50; ¹³ C ₅ -IS	0–100 / 0.33; ¹³ C ₅ -IS	0–100 / 0.33; ¹³ C ₅ -IS	0–100 / 0.33; ¹³ C ₅ -IS
<i>Group 2</i>						
11	0.80–78 / 0.44; ¹³ C ₅ -IS	0.3–25 / 0.17; ¹³ C ₅ -IS	Not measured	Not measured	Not measured	Not measured
12	0.025–100 / 0.027; ¹³ C ₅ -IS	0–50 / 0.057; ¹³ C ₅ -IS	Not measured	Not measured	Not measured	Not measured
13	0.61–122.1 / 0.20; ¹³ C ₅ -IS	0.29–57.7 / 0.10; ¹³ C ₅ -IS	0.29–57.1 / 0.10; ¹³ C ₅ -IS	0.32–64.3 / 0.32; ¹³ C ₅ -IS	0.27–53.4 / 0.09; ¹³ C ₅ -IS	0.30–59.6 / 0.10; ¹³ C ₅ -IS

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14	1–100 / 0.2; ¹³ C ₅ -IS	0.2–20 / 0.2; ¹³ C ₅ -IS	0.2–20 / 0.2; ¹³ C ₅ -IS	0.5–20 / 0.4; ¹³ C ₅ -IS	0.2–20 / 0.2; ¹³ C ₅ -IS	0.5–20 / 0.4; ¹³ C ₅ -IS
15	1–100 / 0.20; ¹³ C ₅ -IS	0.5–50 / 0.44; ¹³ C ₅ -IS	0.5–50 / 0.33; ¹³ C ₅ -IS	0.5–50 / 0.69; ¹³ C ₅ -IS	0.5–50 / 0.53; ¹³ C ₅ -IS	0.5–50 / 0.36; ¹³ C ₅ -IS
16 ⁶	0.7–70 / 0.063, 0.036; ¹³ C ₅ -IS	0.5–50 / 0.71, 0.51; ¹³ C ₅ -IS	0.5–50 / 0.11, 0.21; ¹³ C ₅ -IS	0.5–50 / 0.19, 0.11; ¹³ C ₅ -IS	0.5–50 / 0.25, 0.18; ¹³ C ₅ -IS	0.5–50 / 0.34, 0.44; ¹³ C ₅ -IS

¹ Concentrations are in nmol/L; ¹³C₅-IS, internal standard labeled with 5 ¹³C atoms (purchased from Merck & Cie, Basel, Switzerland, unless noted otherwise); 5,10-methenylTHF, 5,10-methenyltetrahydrofolate; 5-formylTHF, 5-formyltetrahydrofolate; 5-methylTHF, 5-methyltetrahydrofolate; MeFox, pyrazino-s-triazine derivative of 4 α -hydroxy-5-methylTHF; LOD, limit of detection; THF, tetrahydrofolate.

² Internal standard prepared in-house from ¹³C₅-5-methylTHF.

³ Internal standard synthesized in-house (Freisleben et al. J Agric Food Chem 2002;50:4760–8).

⁴ Internal standard obtained from Institute of Food Research, Norwich, United Kingdom.

⁵ Internal standard prepared in-house from ¹³C₅-5-formylTHF.

⁶ Two LOD values are shown because laboratory used daily signal-to-noise ratio to determined LOD.

Supplemental Table 2. Value assignment for folate calibrators¹

Laboratory #	5-MethylTHF	Folic acid	5-FormylTHF	THF	5,10-MethenylTHF	MeFox
10	$\lambda=290$ nm, pH=7.2, $\epsilon=31700^2$	$\lambda=282$ nm, pH=7.2, $\epsilon=27600^2$ ($\lambda=346$ nm, pH=7.2, $\epsilon=7200$)	$\lambda=285$ nm, pH=7.2, $\epsilon=37200^2$	$\lambda=298$ nm, pH=7.2, $\epsilon=25000^2$	$\lambda=348$ nm, pH=0.0, $\epsilon=26500^2$ ($\lambda=288$ nm, pH=0.0, $\epsilon=13500$)	$\lambda=280$ nm, pH=12.3, $\epsilon=19365^2$
<i>Group 1</i>						
1	$\lambda=290$ nm, pH=7.0, $\epsilon=31700^2$	$\lambda=282$ nm, pH=7.0, $\epsilon=27000^3$	Not measured	Not measured	Not measured	Not measured
2	Gravimetric	Gravimetric	Gravimetric	Not measured	Not measured	Gravimetric ⁴
3	Gravimetric ^{5,6}	Gravimetric	Gravimetric ^{5,6}	Gravimetric ^{2,6}	Not measured	Not measured
4	$\lambda=290$ nm, pH=7.0, $\epsilon=31700^2$	$\lambda=282$ nm, pH=8.0, $\epsilon=27600^3$	$\lambda=285$ nm, pH=7.0, $\epsilon=37200^2$	$\lambda=297$ nm, pH=7.0, $\epsilon=28000^3$	$\lambda=350$ nm, pH=7.0, $\epsilon=24900^2$	No measured
5	$\lambda=290$ nm, pH=7.2, $\epsilon=31700^2$	$\lambda=282$ nm, pH=7.2, $\epsilon=27600^2$	$\lambda=285$ nm, pH=7.2, $\epsilon=37200^2$	$\lambda=297$ nm, pH=7.2, $\epsilon=29100^2$	$\lambda=352$ nm, pH=7.2, $\epsilon=25000^2$	Not measured
6	$\lambda=290$ nm, pH=7.2, $\epsilon=31700^2$	$\lambda=282$ nm, pH=7.2, $\epsilon=27600^2$	$\lambda=285$ nm, pH=7.2, $\epsilon=37200^2$	$\lambda=297$ nm, pH=7.2, $\epsilon=29100^2$	$\lambda=352$ nm, pH=7.2, $\epsilon=25000^2$	$\lambda=280$ nm, pH=7.2, $\epsilon=23900^2$
7	$\lambda=290$ nm, pH=7.2, $\epsilon=31700^5$	$\lambda=282$ nm, pH=7.2, $\epsilon=27600^5$ ($\lambda=346$ nm, pH=7.2, $\epsilon=7200$)	$\lambda=285$ nm, pH=7.2, $\epsilon=37200^5$	$\lambda=298$ nm, pH=7.2, $\epsilon=25000^5$	$\lambda=348$ nm, pH=0.3, $\epsilon=26500^5$ ($\lambda=288$ nm, pH=0.3, $\epsilon=13500$)	Obtained from CDC
<i>Group 2</i>						
11	$\lambda=290$ nm, pH=7.2, $\epsilon=31700^3$	$\lambda=282$ nm, pH=7.1, $\epsilon=27600^3$	Not measured	Not measured	Not measured	Not measured
12	$\lambda=290$ nm, pH=7.0, $\epsilon=31700^2$	$\lambda=282$ nm, pH=7.0, $\epsilon=27600^2$	Not measured	Not measured	Not measured	Not measured
13	$\lambda=290$ nm, pH=7.2, $\epsilon=31700^2$	$\lambda=282$ nm, pH=7.2, $\epsilon=27600^2$	$\lambda=285$ nm, pH=7.2, $\epsilon=37200^2$	$\lambda=298$ nm, pH=7.2, $\epsilon=25000^2$	$\lambda=348$ nm, pH=1.0, $\epsilon=26500^2$	$\lambda=280$ nm, pH=12.4, $\epsilon=19365^2$

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14	$\lambda=290$ nm, pH=7.2, $\epsilon=31700^2$	$\lambda=282$ nm, pH=7.2, $\epsilon=27600^2$	$\lambda=285$ nm, pH=7.2, $\epsilon=37200^2$	$\lambda=298$ nm, pH=7.2, $\epsilon=25000^2$	$\lambda=288$ nm, pH=0.0, $\epsilon=13500^2$	$\lambda=280$ nm, pH=13, $\epsilon=19365^2$
15	$\lambda=290$ nm, pH=7.2, $\epsilon=31700^2$	$\lambda=282$ nm, pH=7.2, $\epsilon=27600^2$	$\lambda=285$ nm, pH=7.0, $\epsilon=37200^2$	$\lambda=298$ nm, pH=7.0, $\epsilon=25000^2$	$\lambda=348$ nm, pH=0.3, $\epsilon=26500^2$	$\lambda=280$ nm, pH=12.4, $\epsilon=19365^2$
16	$\lambda=290$ nm, pH=7.2, $\epsilon=31700^2$	$\lambda=282$ nm, pH=7.2, $\epsilon=27600^2$ ($\lambda=346$ nm, pH=7.2, $\epsilon=7200$)	$\lambda=285$ nm, pH=7.2, $\epsilon=37200^2$	$\lambda=298$ nm, pH=7.2, $\epsilon=25000^2$	$\lambda=348$ nm, pH=0.0, $\epsilon=26500^2$ ($\lambda=288$ nm, pH=0.0, $\epsilon=13500$)	Obtained from CDC

¹ For spectrophotometric value assignment, the wavelength (λ), pH, and absorptivity coefficient (ϵ) used is provided; information provided in parenthesis indicates a second wavelength, however results are not used for value assignment; 5,10-methenylTHF, 5,10-methenyltetrahydrofolate; 5-formylTHF, 5-formyltetrahydrofolate; 5-methylTHF, 5-methyltetrahydrofolate; MeFox, pyrazino-s-triazine derivative of 4 α -hydroxy-5-methylTHF; THF, tetrahydrofolate.

² Primary calibrator purchased from Merck & Cie (Basel, Switzerland).

³ Primary calibrator purchased from Sigma-Aldrich.

⁴ Primary calibrator prepared in-house from 5-methylTHF.

⁵ Primary calibrator purchased from Schircks (Jona, Switzerland).

⁶ Concentrations of 5-methylTHF, 5-formylTHF, and THF verified by HPLC with diode array detection using predetermined response factor.

Supplemental Table 3. Published information on method imprecision and spike recovery by laboratory and folate form¹

Laboratory #	Reference	5-MethylTHF	Folic acid	MeFox	5-FormylTHF	THF	5,10-MethenylTHF
Imprecision, %							
10	6, 8	2.6–3.4	6.2–8.4	3.3–4.5	3.1–11.0	7.0–9.6	5.4–7.5
<i>Group 1</i>							
2	16	4.1–5.1	6.1–11.9	5.7–7.4	5.5–10.5	n/a	n/a
3	17	3.0	4.0	n/a	3.0	5.0	n/a
4	14, 15	≤9.0	≤6.5	n/a	n/a	n/a	n/a
5	18, 19	2.2–2.6	4.7–6.1	n/a	3.6–4.4	15.6–20	7.1–10.4
7	20	3.8–7.9	4.8–19.5	n/a	6.7–21.3	5.5–15.5	7.2–9.0
Spike recovery, %							
10	6, 8	93–105	92–105	92–104	84–96	93–103	98–113
<i>Group 1</i>							
2	16	103–108	86–90	82–94	91–98	n/a	n/a
3	17	95	105	n/a	96	105	n/a
4	14, 15	98.7–102.5	96.6–104.7	n/a	n/a	n/a	n/a
5	18, 19	98.2	98.2	n/a	102.3	82.3	110.8
7	20	97.4–106.2	91.2–103.9	n/a	94.3–101.1	91.2–99.4	94.6–101.3

¹ Only laboratory 10 (CDC) and laboratories 2, 3, 4, 5, and 7 (group 1) published their independently-developed methods; n/a, information not available in published article; 5-formylTHF, 5-formyltetrahydrofolate; 5-methylTHF, 5-methyltetrahydrofolate; 5,10-methenylTHF, 5,10-methenyltetrahydrofolate; MeFox, pyrazino-s-triazine derivative of 4 α -hydroxy-5-methylTHF; THF, tetrahydrofolate.

Supplemental Table 4. Concentrations of MeFox in NIST SRM 1955 by laboratory¹

Laboratory #	Concentration \pm SD, nmol/L		
	Level 1	Level 2	Level 3
10	1.69 \pm 0.15	3.74 \pm 0.29	6.25 \pm 0.06
<i>Group 1</i>			
2	1.99 \pm 0.01	3.65 \pm 0.16	5.74 \pm 0.11
6	1.18 \pm 0.06	2.54 \pm 0.15	3.97 \pm 0.11
7	2.31 \pm 0.62	3.80 \pm 0.06	6.36 \pm 0.50
<i>Mean \pm SD</i>	<i>1.83 \pm 0.58</i>	<i>3.33 \pm 0.69</i>	<i>5.36 \pm 1.24</i>
<i>Group 2</i>			
13	1.80 \pm 0.02	3.93 \pm 0.02	6.08 \pm 0.25
14	1.48 \pm 0.08	3.51 \pm 0.78	5.49 \pm 0.06
15	1.8 \pm 0.01	3.97 \pm 0.35	6.92 \pm 0.21
16	2.04 \pm 0.14	4.08 \pm 0.32	6.81 \pm 1.30
<i>Mean \pm SD</i>	<i>1.78 \pm 0.23</i>	<i>3.87 \pm 0.25</i>	<i>6.33 \pm 0.67</i>

¹ Values are means \pm SD, n = 2 (i.e., duplicate analysis); laboratories #1, #3, #4, #5, #11, and #12 did not measure pyrazino-s-triazine derivative of 4 α -hydroxy-5-methylTHF (MeFox).

Supplemental Table 5. Concentrations of 5-methyltetrahydrofolate and folic acid in NIST SRM 3949 by laboratory¹

Laboratory #	Concentration \pm SD, nmol/L					
	5-Methyltetrahydrofolate			Folic acid		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
10	15.0 \pm 0.35	46.1 \pm 0.64	29.8 \pm 0	1.17 \pm 0.04	10.3 \pm 0.14	7.59 \pm 0.02
<i>Group 1</i>						
1	16.8 \pm 0.56	52.7 \pm 0.63	33.8 \pm 2.36	0.70 \pm 0.03	6.10 \pm 0.06	3.70 \pm 0.27
2	15.2 \pm 0.21	37.7 \pm 0.64	30.9 \pm 1.13	1.26 \pm 0.06	9.20 \pm 0.88	6.21 \pm 0.53
3	16.8 \pm 1.20	57.3 \pm 1.77	40.9 \pm 6.36	1.55 \pm 0.21	9.65 \pm 2.62	4.00 \pm 2.40
4	14.8 \pm 1.42	42.7 \pm 2.33	31.1 \pm 0.70	2.12 \pm 0.003	13.4 \pm 2.65	9.06 \pm 0.07
5	14.7 \pm 0.48	47.3 \pm 3.37	30.3 \pm 0.67	0.38 \pm 0.02	4.09 \pm 0.68	3.08 \pm 0.19
6	15.9 \pm 0.69	50.5 \pm 0.43	31.6 \pm 0.78	0.76 \pm 0.09	6.75 \pm 0.08	4.25 \pm 0.34
7	15.0 \pm 0.21	46.7 \pm 2.59	30.2 \pm 0.74	<LOQ ²	6.41 \pm 0.35	4.30 \pm 0.01
<i>Mean \pm SD</i>	<i>15.6 \pm 0.91</i>	<i>47.8 \pm 6.47</i>	<i>32.7 \pm 3.82</i>	<i>1.13 \pm 0.64</i>	<i>7.94 \pm 3.06</i>	<i>4.94 \pm 2.06</i>
<i>Group 2</i>						
11	15.9 \pm 0.62	44.9 \pm 1.82	33.7 ²	1.68 \pm 0.03	8.45 \pm 0.60	5.85 ³
12	15.1 \pm 0.35	47.7 \pm 2.15	35.6 \pm 0.15	1.50 \pm 0.08	7.59 \pm 0.90	5.38 \pm 1.04
13	14.8 \pm 0.30	48.3 \pm 1.80	30.9 \pm 0.57	0.77 \pm 0	5.93 \pm 0.41	4.11 \pm 0.30
14	15.6 \pm 0.35	47.5 \pm 0	30.9 \pm 0.35	1.08 \pm 0.26	8.13 \pm 0.51	5.55 \pm 0.28
15	15.4 \pm 0.57	47.5 \pm 0.85	32.7 \pm 0.28	1.18 \pm 0.04	7.09 \pm 0.21	5.26 \pm 0.30
16	13.7 \pm 0.26	43.5 \pm 1.03	29.1 \pm 1.33	1.73 \pm 0.12	7.09 \pm 1.22	4.94 \pm 0.20
<i>Mean \pm SD</i>	<i>15.1 \pm 0.78</i>	<i>46.6 \pm 1.91</i>	<i>32.2 \pm 2.33</i>	<i>1.32 \pm 0.38</i>	<i>7.38 \pm 0.90</i>	<i>5.18 \pm 0.61</i>

¹ Values are means \pm SD, n = 2 (i.e., duplicate analysis).² Laboratory reported results as <LOQ (<1 nmol/L).³ Laboratory provided only a single result.

Supplemental Table 6. Concentrations of MeFox, 5-formylTHF, THF, and 5,10-methenylTHF in NIST SRM 3949 by laboratory¹

Laboratory #	Concentration \pm SD, nmol/L					
	Level 1	MeFox Level 2	Level 3	5-FormylTHF Level 3	THF Level 3	5,10-MethenylTHF Level 3
10	1.21 \pm 0.08	1.70 \pm 0.02	1.92 \pm 0.06	5.30 \pm 0.01	0.55 \pm 0	0.57 \pm 0.02
<i>Group 1</i>						
2	1.49 \pm 0.04	1.94 \pm 0.04	2.57 \pm 0.01	7.07 \pm 0.33	n.m.	n.m.
3	n.m.	n.m.	n.m.	4.15 \pm 0.35	<LOD	n.m.
4	n.m.	n.m.	n.m.	2.10 \pm 0.20	<LOD	1.23 \pm 0.23
5	n.m.	n.m.	n.m.	7.43 \pm 0.21	0.90 ²	0.70 \pm 0.22
6	0.82 \pm 0.03	1.34 \pm 0.04	1.29 \pm 0.05	4.54 \pm 0.08	0.95 \pm 0.12	0.47 \pm 0.04
7	1.15 \pm 0.11	2.02 \pm 0.16	1.79 \pm 0.01	4.89 \pm 0.06	<LOQ ²	<LOQ ³
<i>Mean \pm SD</i>	<i>1.15 \pm 0.34</i>	<i>1.77 \pm 0.37</i>	<i>1.88 \pm 0.65</i>	<i>5.03 \pm 1.98</i>	<i>0.93 \pm 0.04</i>	<i>0.80 \pm 0.39</i>
<i>Group 2</i>						
13	1.32 \pm 0.03	2.07 \pm 0.12	2.22 \pm 0.01	6.83 \pm 0.02	0.82 \pm 0.09	0.17 \pm 0.01
14	1.37 \pm 0.16	2.45 \pm 0.37	1.98 \pm 0.23	4.82 \pm 0.40	1.06 \pm 0.28	<LOD
15	1.39 \pm 0.23	2.17 \pm 0.02	2.42 \pm 0.08	15.7 \pm 1.13	1.11 \pm 0.42	<LOD
16	<LOD	1.31 ²	0.86 ⁴	11.4 \pm 2.01	1.55 \pm 1.24	<LOD
<i>Mean \pm SD</i>	<i>1.36 \pm 0.04</i>	<i>2.00 \pm 0.49</i>	<i>1.87 \pm 0.70</i>	<i>9.69 \pm 4.86</i>	<i>1.14 \pm 0.30</i>	<i>0.17</i>

¹ Values are means \pm SD, n = 2 (i.e., duplicate analysis); laboratories 1, 11, and 12 did not measure pyrazino-s-triazine derivative of 4 α -hydroxy-5-methylTHF (MeFox), 5-formyltetrahydrofolate (5-formylTHF), tetrahydrofolate (THF), and 5,10-methenyltetrahydrofolate (5,10-methenylTHF); n.m., not measured.

² Laboratory reported 1 result <LOD (<0.9 nmol/L) and 1 result <LOQ (<1 nmol/L).

² Laboratory reported results as <LOQ (<1 nmol/L).

⁴ Laboratory provided 1 result >LOD and 1 result <LOD (<0.44 nmol/L); result <LOD was not used to calculate the mean.

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12	19.8 ± 1.23	18.9 ± 1.15	3.95 ± 0.52	4.18 ± 0.55	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n/a
13	20.7 ± 0.33	20.0 ± 0.87	2.41 ± 0.09	2.48 ± 0.26	5.05 ± 0.21	5.51 ± 0.21	5.92 ± 0.13	5.89 ± 0.14	3.54 ± 0.35	3.81 ± 0.46	3.83 ± 0.34	4.46 ± 0.18	14.2 ± 0.51
14	18.9 ± 0.93	19.1 ± 0.59	3.37 ± 0.13	3.58 ± 0.12	4.89 ± 0.32	4.90 ± 0.15	4.42 ± 0.27	3.13 ± 0.30	6.13 ± 1.13	5.05 ± 0.35	4.65 ± 0.23	6.57 ± 0.63	14.7 ± 0.81
15	19.5 ± 0.10	19.7 ± 0.45	3.37 ± 0.09	3.46 ± 0.13	5.44 ± 0.12	5.84 ± 0.32	11.6 ± 1.36	12.7 ± 1.52	5.51 ± 0.13	5.70 ± 0.26	3.01 ± 0.14	4.04 ± 0.25	22.4 ± 1.47
16	19.6 ± 1.03	18.8 ± 1.73	2.48 ± 0.18	2.66 ± 0.20	5.23 ± 0.54	6.27 ± 0.56	8.75 ± 1.31	6.50 ± 0.87	14.0 ± 3.70	14.4 ± 3.41	5.02 ± 0.59	7.39 ± 1.00	28.3 ± 3.36
<i>Mean ± SD</i>	<i>19.8 ± 0.62</i>	<i>19.4 ± 0.51</i>	<i>3.36 ± 0.83</i>	<i>3.49 ± 0.82</i>	<i>5.15 ± 0.24</i>	<i>5.63 ± 0.58</i>	<i>7.67 ± 3.18</i>	<i>7.06 ± 4.04</i>	<i>7.30 ± 4.60</i>	<i>7.24 ± 4.84</i>	<i>4.13 ± 0.90</i>	<i>5.62 ± 1.62</i>	

¹ Values are means ± SD, n = 4 (i.e., duplicate analysis on 2 days); “single” means that individual calibrator was measured; “mix” means that a mixture of all 6 calibrators was measured; 5-formylTHF, 5-formyltetrahydrofolate; 5-methylTHF, 5-methyltetrahydrofolate; 5,10-methenylTHF, 5,10-methenyltetrahydrofolate; MeFox, pyrazino-s-triazine derivative of 4 α -hydroxy-5-methylTHF; THF, tetrahydrofolate; n.m., not measured.

² Non-methyl was calculated as the sum of 5-formylTHF, THF, and 5,10-methenylTHF in the calibrator mix for those laboratories who measured all 3 folate forms; n/a, not applicable for laboratories who did not measure all 3 folate forms.

Supplemental Table 8. Difference in folate concentration measured by each laboratory relative to the CDC laboratory¹

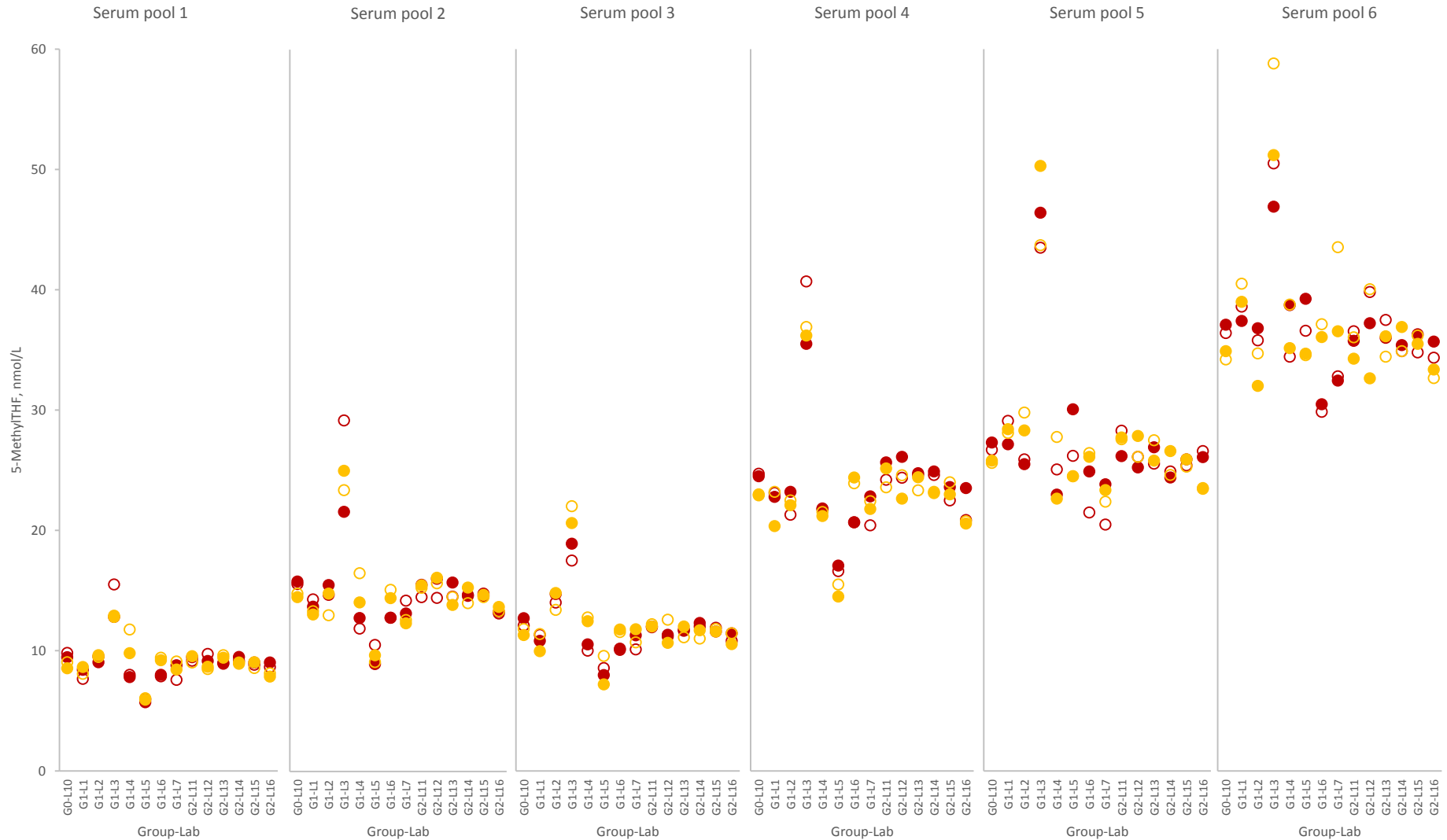
Laboratory #	Relative difference to CDC laboratory, %						
	5-MethylTHF	Folic acid	Folic acid ²	MeFox	5-FormylTHF	THF	5,10-MethenylTHF
<i>Group 1</i>							
1	-3.5	-63	-43	Not measured	Not measured	Not measured	Not measured
2	1.8	-25	18	9.3	16	Not measured	Not measured
3	59	-4.7	52	Not measured	-1.5	25	Not measured
4	-4.3	41	125	Not measured	-6.7	*	9.3
5	-23	-61	-38	Not measured	35	-15	4.5
6	-7.3	-41	-7.3	-32	-15	-0.6	2.7
7	-8.5	-47	-19	1.7	-10	-19	20
<i>Group 2</i>							
11	1.4	-13	39	Not measured	Not measured	Not measured	Not measured
12	0.4	-28	14	Not measured	Not measured	Not measured	Not measured
13	-0.6	-49	-19	8.0	28	-6.8	-14
14	-1.7	-35	3.2	-4.9	-12	33	8.7
15	-2.5	-33	7.0	15	148	37	-25
16	-8.1	-16	36	26	111	345	-18

¹ Relative difference calculated as the mean from 6 serum pools for 5-methylTHF, folic acid, and MeFox; relative difference shown for spiked serum sample for 5-formylTHF, THF, and 5,10-methenylTHF; 5-methylTHF, 5-formylTHF, 5-formyltetrahydrofolate; 5-methyltetrahydrofolate; 5,10-methenylTHF, 5,10-methenyltetrahydrofolate; MeFox, pyrazino-s-triazine derivative of 4 α -hydroxy-5-methylTHF; THF, tetrahydrofolate.

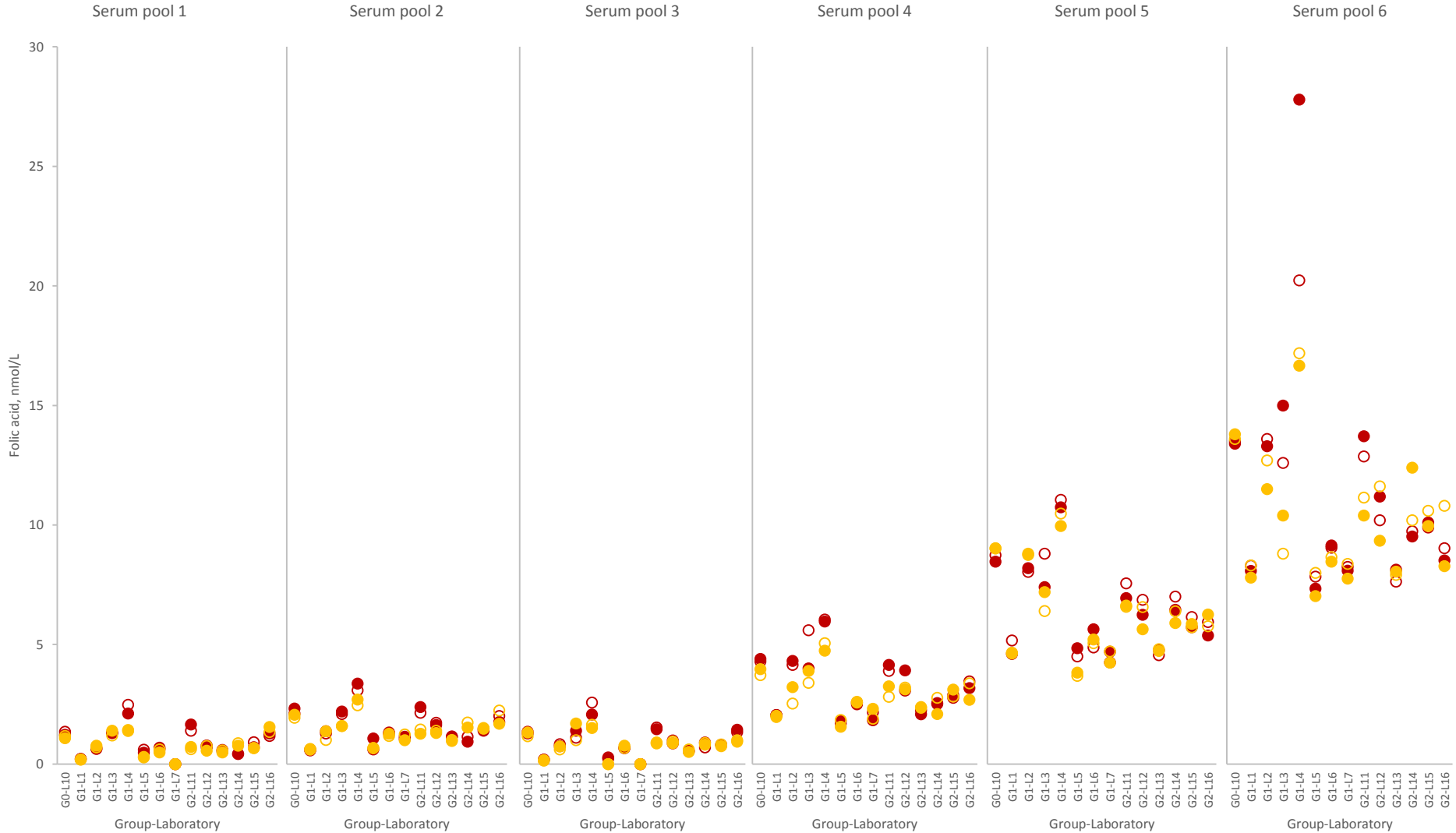
² Results after laboratory 10 corrected folic acid calibration bias.

* Relative difference could not be calculated because laboratory reported <LOD (<0.5 nmol/L) results.

Supplemental Figure 1. Serum 5-methyltetrahydrofolate concentrations by laboratory for 6 serum pools and 4 measurements each. Each circle represents one measurement : red closed circle, day 1 replicate 1; red open circle, day 1 replicate 2; gold closed circle, day 2 replicate 1; gold open circle, day 2 replicate 2. G0-L10, CDC laboratory. G1-L1 through G1-L7, group 1 laboratories. G2-L11 through G2-L16, group 2 laboratories.



Supplemental Figure 2. Serum folic acid concentrations by laboratory for 6 serum pools and 4 measurements each. Each circle represents one measurement: red closed circle, day 1 replicate 1; red open circle, day 1 replicate 2; gold closed circle, day 2 replicate 1; gold open circle, day 2 replicate 2. G0-L10, CDC laboratory. G1-L1 through G1-L7, group 1 laboratories. G2-L11 through G2-L16, group 2 laboratories.



Supplemental Figure 3. Serum MeFox concentrations by laboratory for 6 serum pools and 4 measurements each. Each circle represents one measurement: red closed circle, day 1 replicate 1; red open circle, day 1 replicate 2; gold closed circle, day 2 replicate 1; gold open circle, day 2 replicate 2. G0-L10, CDC laboratory. G1-L1 through G1-L7, group 1 laboratories. G2-L11 through G2-L16, group 2 laboratories.

